

REMARKS/ARGUMENTS

Applicants thank the Examiner and Primary Examiner for the telephonic interview relating to this application that was conducted on January 21, 2004.

The Invention

The invention is a functional origin of replication comprising the replication sequences, genes and proteins that make possible the replication of extrachromosomal elements in *Fusobacterium* species. The invention further comprises a series of plasmids bearing this origin that replicate in *Fusobacterium* species and a shuttle vector that replicates in *Escherichia coli* as well as *Fusobacterium*. Finally the invention provides, for the first time, methods for the transformation of *Fusobacterium* species with plasmids.

Status of the Claims

Claims 1-63 are pending in the application.

Claims 1-4, 11, 15-20, 26, 37-40, 49, 50, 55-57, 59, and 62 are allowed.

Claims 6, 9, 14, 22, 23, 28, 29, 42, 43, 60, and 63 are objected to.

Claims 5, 7, 8, 10, 12, 13, 21, 24, 25, 27, 30-36, 41, 44-48, 51-54, 58, and 61 are rejected.

Support for Amendments to the Claims

Support for the amendment to claim 5 is found in the specification on page 7, lines 16-18 wherein it is stated that: "The terms 'isolated'...refer to material that substantially or essentially free from components which normally accompany it as found in its native state.", Support is also found on page 7, lines 21-23 wherein it is stated that : "...an isolated repA nucleic acid is separated from open reading frames that flank the repA gene...". Further support for the amendment is found on page 19, lines 27-30 wherein it is stated that: "the RepA polypeptides encoded by the nucleic acids of the invention... are at least about 90% identical to an amino acid sequence of SEQ ID NO:1...". No new matter is added.

Support for the amendment to claim 12, claim 21, claim 27, and claim 41 is found on page 19, lines 27-30 wherein it is stated that:"the RepA polypeptides encoded by the nucleic acids of the invention... are at least about 90% identical to an amino acid sequence of SEQ ID NO:1...". No new matter is added.

Support for the amendment to claim 33 is found on page 3, lines 22-28 wherein it is stated that:"the present invention provides an isolated nucleic acid molecule comprising a DNA fragments of plasmid pFN1 or plasmid pFN2. In one embodiment, the isolated nucleic acid molecule comprises a 2.36 kb DNA fragment generated by cleaving plasmid pFN1 with restriction endonucleases *AvrII* and *ScaII*. In another embodiment, the isolated nucleic acid molecule comprises a 0.9 kb DNA fragment generated by cleaving plasmid pFN2 with restriction endonucleases *HincII* and *HpaII*.", and throughout the specification. No new matter is added.

Support for the amendment to claim 34 is found on page 5, line 2 wherein it is stated that:"pFN1 that has a GenBank Accession No. AF159249", and on page 45, line 16 wherein the sequence of pFN1 is designated as SEQ ID NO:6. No new matter is added.

Support for the amendment to claim 33, claim 35, and claim 36 is found in the deposit receipt from the American Type Culture Collection and in the verified statement of Susan Kinder Haake corroborating that the stains deposited with the American Type Culture Collection are identical to the strains described in the specification. No new matter is added.

Support for the amendment to claim 48 is found on page 48, line 8, wherein pHS17 is identified as SEQ ID NO:15. No new matter is added.

Support for the amendment to claim 57 is found on page 38, line 6, wherein the term *Escherichia coli* is recited. No new matter is added.

Support for the amendment to claim 63 is found in original claim 63 and in Example 5 on pages 40-42 wherein a method for transforming *E. coli* with the shuttle plasmid pHS17 is disclosed. No new matter is added.

Allowable Subject Matter

Claims 1-4, 11, 15-20, 26, 37-40, 49, 50, 55-57, 59, and 62 are allowed.

Claims 6, 9, 14, 22, 23, 28, 29, 42, 43, and 60 are objected to as being dependent on a rejected base claim. The Examiner has indicated that claims 6, 9, 14, 22, 23, 28, 29, 42, 43, and 60 would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

Response to Claim Objections

Claim 63 is objected to as being identical to claim 62. Claim 63 is amended to recite *E. coli* in place of *F. nucleatum*, thereby correcting the claim.

Response to Claim Rejections under 35 U.S.C. §112, first paragraph: written description

Strain Deposits

Claims 10, 33-36, 48 and 60 are rejected under 35 U.S.C. §112, first paragraph as containing subject matter which was allegedly not described in a way so as to enable one skilled in the art to make and/or use the invention. The Examiner is requesting a deposit of the strains harboring plasmids pFN1, pFN2, pFN3 and pHS17.

In a telephonic interview conducted on January 21, 2004 the Examiner agreed that a strain deposit was not necessary for the plasmids pFN1 (SEQ ID NO:6) and pHS17 (SEQ ID NO:15) since the specification provides a full nucleotide sequence for each of these plasmids. However, the Examiner maintained that a deposit of strains is still required to satisfy the written description requirement for pFN2 and pFN3, since full nucleotide sequence for these plasmids is not provided by the specification.

To address the Examiner's concerns, the Applicants submit herewith copies of the deposit receipts for strains harboring the plasmids pFN2 and pFN3. The Applicants further submit a verified statement of Dr. Susan Kinder Haake, corroborating that the biological material described in the specification as filed is the same as that deposited in the depository, stating that the deposited material is identical to the biological material described in the specification and was in the Applicant's possession at the time the application was filed. Applicants have also amended the specification herein to recite the complete name and address of the depository.

Applicants' undersigned agent of record further states that a cell line harboring pFN2 (PTA-5816) and a cell line harboring pFN3 (PTA-5815) were deposited on February 13, 2004 with an International Depository Authority under the provisions of the Budapest Treaty and pursuant to 37 C.F.R. § 1.801-1.809 and the viability of the biological material was tested on February 20, 2004 (see deposit receipt and viability statement submitted herewith) and

(a) during the pendency of this application, access to the deposits will be afforded to the Commissioner upon request;

(b) all restrictions upon public access to the deposited biological material will be irrevocably removed upon granting of a patent issued from the present patent application;

(c) the deposits will be maintained in a public depository for a period of at least thirty years from the date of deposit or for the enforceable life of the patent or for a period of five years after the date of the most recent request for furnishing of a sample of the deposited biological material, whichever is the longest;

(d) the deposits will be replaced if they should become nonviable or non-replicable.

In view of the fact that the strains have been properly deposited with a recognized International depository, Applicants respectfully request reconsideration of the written description rejection based on the requirement for strain deposits.

Possession of the Claimed Invention

Claims 5, 7, 8, 12, 13, 21, 24, 25, 27, 30-32, 41, 44-47, 51-54, 58, and 61 are rejected for allegedly containing subject matter that is not described in the specification in such a way as to convey to one skilled in the art that the inventors had possession of the claimed invention at the time the application was filed. The Applicants respectfully disagree.

The Applicants presently claim functional proteins, and nucleic acids encoding functional proteins, that comprise 90% sequence identity to SEQ ID NO:1. Support for this claim is provided by the accompanying declaration of Susan Kinder Haake under 35 U.S.C. §1.132.

The Examiner alleges that the Applicant claims proteins by function only, without any disclosed or known correlation between elements and their function, that the prior art does not provide sufficient information to overcome the alleged deficiencies of the specification, and that the prior art allegedly demonstrates that predicting function based on sequence homology is unpredictable.

To exemplify the assertion that sequence homology is an unpredictable indicator of protein function, the Examiner cites Everett *et al.* (Nat. Gen. 17:411-422). Based on sequence homology to known ion transport proteins, Everett *et al.* predicted that the Pendred gene product, PDS, would function as Sulphate ion transporter. Apparently, it was later discovered that PDS was in fact actually a chloride and iodide ion transporter.

Adequate written description of the invention may be shown by any description of sufficient, relevant, identifying characteristics so long as a person skilled in the art would recognize that the inventor had possession of the claimed invention (MPEP 2163(3)(a)).

The Applicants submit that an adequate written description exists for claiming a RepA protein with 90% sequence identity to SEQ ID NO:1. Indeed, as disclosed in the declaration of Susan Kinder Haake under 35 U.S.C. §1.132 (henceforth the "132 declaration") Rep proteins are known in the art to exhibit conserved function across a wide range of sequence divergence.

The del Solar paper (Exhibit A) which accompanies the 132 declaration, discloses features common to replication initiator proteins, like RepA, that are functional in theta-replicating plasmids of the iteron-containing class. Some of the common identifying features of proteins belonging to this class include: the presence of directly repeated iteron sequences in the origin region arranged as tandem repeats that are multiples of 11 base pairs (Exhibit A, page 437, column 1 paragraph 2), an AT rich region adjacent to the origin of replication (Exhibit A, page 435, column 2, paragraph 5), and one or more DnaA boxes (Exhibit A, column 2, paragraph 5). These common structural features are also present in the origin region of pFN1, upstream of the RepA protein ORF (*see e.g.*, specification Figure 2B and corresponding legend page 6, lines 18-21; page 37, lines 31-34; and page 38, lines 1-6). As can be seen in Figure 2 of Exhibit A,

related replication initiator proteins may exhibit more than 75% amino acid sequence divergence and still be readily recognizable as replication initiator proteins.

The written description requirement may be met through sufficient description of a representative number of species (MPEP 2163(a)(ii)). A representative number of species means that the species which are adequately described are representative of the entire genus. Whether a satisfactory disclosure of a representative number of species has been achieved depends on whether one of skill in the art would recognize that the Applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed. The description of a representative number of species does not require the description to be of such specificity that it would provide individual support for each species that the genus embraces (MPEP 2163(a)(ii)).

Exhibit A presents a comparative amino acid sequence alignment and analysis of a representative sampling of replication initiator proteins from theta-replicating plasmids of the iteron-containing class. The analysis, while not addressed specifically to the pFN1 replicon, is nonetheless applicable to the pFN1 replicon as the Rep proteins analyzed in Exhibit A are all functional components of theta-replicating plasmids with origin structures comparable to that of the fusobacterial plasmid pFN1.

Figure 2 of Exhibit A shows that related replication initiator proteins may share only small amounts of sequence identity. Indeed, in the most divergent plasmids, pPS10 and RK6, Rep proteins share only about 24% sequence identity. The calculations and analysis of the sequence identity shared by pPS10 and RK6, that is provided by Dr. Kinder Haake in the 132 declaration, underscores the fact that only a minimal amount of sequence identity is required for *accurate* identification of replication initiator proteins. In fact, the 24% sequence identity between pPS10 and RK6 observable in the figure, only represents the sequence identity between aligned regions of the two proteins. When the entire amino acid sequences comprising the two proteins are compared, the sequence identity between the overall proteins is even less than 24%.

Furthermore, it should be noted that the RepA protein of the invention was identified as a replication initiator protein, at least in part, by alignment of the RepA amino acid

sequence with several other known replication initiator proteins that share only 10%-19% sequence identity with RepA (page 37, lines 27-32).

Thus, in contrast to the inaccurate identification of ion transport proteins disclosed by Everett *et al.* and cited by the Examiner, the prior art directly concerned with replication initiator proteins indicates that only minimal sequence identity is required for one of skill in the art to correctly identify a replication initiator protein.

In addition, because replication initiator proteins from theta-replicating plasmids of the iteron-containing class perform the same function, by way of a similar mechanism that is conserved across species in the bacterial kingdom (*see e.g.*, Exhibit A, page 436, column 2, and page 437, column 1, paragraph 2), closely related proteins from plasmids of the same bacterial species can complement each other's function *in vivo*.

Exhibit B, which also accompanies the 132 declaration, demonstrates the principle of *in vivo* complementation experimentally. Exhibit B discloses the results of experiments wherein related lactococcal replication initiator proteins are able to complement each other's function even though they share only 60%-80% sequence identity (*see e.g.*, abstract and also discussion section, page 1297). These experiments underscore the fact that closely related replication initiator proteins with *significantly less* than 90% sequence identity are functionally equivalent.

In light of the above arguments, one of skill in the art would expect *Fusobacterium* RepA proteins with as much as 90% sequence identity to be functionally equivalent to the repA protein of the invention. Thus, in view of the disclosure of the amino acid sequence of *Fusobacterium* RepA protein (SEQ ID NO:1), one of skill in the art would accept that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus.

Therefore, because replication initiator proteins are known in the art to exhibit conserved function over a wide range of sequence divergence, one of skill in the art would conclude that disclosure of the RepA species of the invention is representative of RepA proteins with 90% sequence identity to SEQ ID NO:1 that are functional in *F. nucleatum*. Thus, the

disclosure satisfies the written description requirement under 35 U.S.C. §112. In light of the above arguments, the Applicants respectfully request reconsideration of the rejection.

Response to Claim Rejections under 35 U.S.C. §112, first paragraph: enablement

Claims 5, 7, 8, 12, 13, 21, 24, 25, 27, 30-32, 41, 44-47, 51-54, 58, and 61 are rejected for allegedly containing subject matter that does not enable one of skill in the art to make and use the invention commensurate with the scope of the claims.

As noted above in the response to the written description rejection, the Applicants presently claim functional proteins, and nucleic acids encoding functional proteins, that comprise 90% sequence identity to SEQ ID NO:1. Arguments that this claim is enabled by the disclosure are provided in the accompanying declaration of Susan Kinder Haake under 35 U.S.C. §1.132.

The Examiner alleges that one of skill in the art could not make and use the invention from the disclosures in the specification coupled with the information known in the art without undue experimentation. The Examiner specifically alleges that the claims are directed to a "function-without-structure" protein, that the scope of the claims is very broad, that predicting function based on homology is an unpredictable art, and that therefore the disclosure represents an invitation to experiment rather than an enabled invention.

As noted by the Examiner, the question of whether undue experimentation is required is not based on a single factor, but rather is a conclusion reached by weighing many factors (MPEP 2164.01(a)). These factors include the nature of the invention, the scope of the invention, the level of skill in the art and the level of predictability of the art. The essence of the Examiner's allegation is that the state of the art, as exemplified by the Examiner's citation of Everett *et al.* is unpredictable at best, and therefore the skilled artisan would be required to carry out a great deal of undue experimentation to identify the claimed proteins. The Applicants respectfully disagree.

The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation (MPEP 2164.01). The test of whether undue experimentation is required is not merely quantitative, since a considerable amount of experimentation is permissible. Rather, the question is whether the specification provides a

reasonable amount of guidance with respect to the direction in which the experimentation should proceed (MPEP 2164.06). Furthermore, as long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement under 35 U.S.C. §112 is satisfied (MPEP 2164.01(a)).

Replication initiator proteins, like RepA, from theta-replicating plasmids of the iteron-containing class are known in the art to exhibit conserved function across a wide range of sequence divergence. Indeed, RepA proteins can be correctly identified as such when they share as little as 24% sequence identity with a reference sequence. Moreover, the 132 declaration and the accompanying Exhibit B provide evidence that closely related RepA proteins with as little as 60%-80% sequence identity can complement each other's function *in vivo*. Therefore the art of identifying replication initiator proteins from amino acid sequence is highly predictable. Furthermore, as evidenced by the 132 declaration of Dr. Kinder Haake, this predictability is known and accepted in the art.

Thus, contrary to the art applicable to ion transport proteins like the Pendred gene product studied by Everette *et al.*, and cited by the Examiner, the identification of replication initiator proteins from amino acid sequence is a highly predictable art.

Undue experimentation is a conclusion reached by weighing many factors. These factors include the nature of the invention, the scope of the invention, the level of skill in the art, the state of the prior art, the level of predictability of the art, and the amount of direction provided by the inventor (MPEP 2164.01(a)).

The specification provides the amino acid sequence of the RepA protein from the pFN1 plasmid of *Fusobacterium nucleatum* (SEQ ID NO:1) and methods determining and analyzing percent sequence identity (*see e.g.*, page 12, lines 9-34, and pages 13-14). Since the art of identifying replication initiator proteins from sequence comparison is predictable, the disclosure of the RepA protein sequence (SEQ ID NO:1) coupled with the knowledge in the art is sufficient to permit those skilled in the art to make and use the invention.

In conclusion, the art of identifying functional replication initiator proteins from sequence identity is a predictable art. The Applicants have provided a sequence of the RepA

protein and a method for determining sequence identity. Therefore, it would not require undue experimentation to obtain RepA proteins with 90% sequence identity to SEQ ID NO:1. Accordingly, it would not require undue experimentation to make and use the invention commensurate with the scope of the claims.

Reconsideration of the rejection is respectfully requested.

Response to Claim Rejections under 35 U.S.C. §112, second paragraph: indefiniteness

Claims 9 and 33 are rejected under 35 U.S.C. §112, second paragraph as being indefinite for allegedly failing to point out and distinctly claim the subject matter which the Applicant regards as the invention.

Claims 9 and 33 are amended to address the Examiner's concerns.

Reconsideration of the rejection is respectfully requested.

Response to Claim Rejections under 35 U.S.C. §102(b)

Claims 5, 7, and 8 are rejected as allegedly being anticipated by McKay *et al.* (Plasmid 33:15-25 (1995)). In his rejection the Examiner states that McKay *et al.* teach an isolated nucleic acid in the form of a naturally occurring plasmid which encodes a protein with 97.2% sequence identity to SEQ ID NO:1.

In a telephonic interview conducted on January 21, 2004 the Examiner clarified his rejection stating that the meaning of the term "isolated" as used in the specification, could refer to an isolated plasmid, and did not necessarily suggest that the nucleic acid encoding Rep A protein was *separated* from other open reading frames with which it coexists in nature.

Claims 5, 7 and 8 are amended to clarify that the Rep A nucleic acid is separated from open reading frames that flank the rep A nucleic acid in its native state. In light of this amendment the Applicants respectfully request reconsideration of the rejection.

CONCLUSION

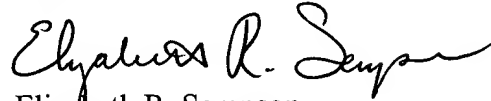
In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

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Amdt. dated April 7, 2004
Reply to Office Action of October 7, 2003

PATENT

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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